DP Barcode: D319265 MRID No.: 465915-06

DATA EVALUATION RECORD
28-DAY WHOLE SEDIMENT *Leptocheirus plumulosus* TOXICITY TEST

1. **CHEMICAL:** Cyfluthrin PC Code: 128831

2. **TEST MATERIAL:** [^{14}C]Cyfluthrin Radiochemical Purity: 99%

3. **CITATION:**

Authors: Putt, A.E.
Title: Cyfluthrin – Toxicity to Estuarine Amphipods (*Leptocheirus plumulosus*) During a 28-Day Sediment Exposure.
Study Completion Date: June 29, 2005
Laboratory: Springborn Smithers Laboratories
790 Main Street
Wareham, MA 02571-1037
Sponsor: Pyrethroid Working Group
Beveridge & Diamond
1350 I Street NW
Washington, DC 20005
Laboratory Report ID: 13656.6116
MRID No.: 465915-06

4. **REVIEWED BY:** Amanda Solliday, Biologist, OPP/EFED/ERB 5

Signature: **Date:** 02/24/11

REVIEWED BY: Justin Housenger, Biologist, OPP/EFED/ERB 5

Signature: **Date:** 02/24/11

5. **APPROVED BY:** Keith Sappington, Senior Advisor, OPP/EFED/ERB 5

Signature: **Date:** 02/24/11

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Leptocheirus plumulosus*
Age of Test Organism: Neonate
Definitive Test Duration: 28 days
Study Method: Static renewal
Type of Concentrations: Mean-measured sediment and pore water (total radioactive residues)

7. CONCLUSIONS:

The 28-day sediment toxicity study of cyfluthrin to estuarine amphipods (*Leptocheirus plumulosus*) was conducted under a static renewal system in which the overlying water was renewed three times weekly. The endpoints assessed were survival and growth.

The nominal spiked sediment concentrations were 0 for the negative and solvent (acetone) controls, 1.9, 5.6, 17, 50, 150, and 450 ug a.i/kg sediment. Measured concentrations at Day-0 (excluding controls) were 1.7, 4.2, 15, 45, 77, and 320 ug a.i/kg sediment, respectively and at test termination on Day-28 were measured at 1.1, 3.5, 11, 26, 44, and 270 ug a.i/kg, respectively representing a decrease of 57 – 84% of Day-0 measurements. Mean measured concentrations in the test were defined as 1.4, 3.8, 13, 35, 60, and 290 ug a.i/kg sediment, respectively.

For the lowest treatment level (1.9 ug a.i/kg nominal), the pore water concentration could not be detected on days 0 and 28. Mean measured pore water concentration for the remaining levels in ascending order were 0.09, 0.25, 0.64, 2.3, and 6.9 ug a.i/L, respectively. This prohibited a statistical analysis of an EC_{50} for growth based on measured pore water concentrations from being calculated due to an undefined concentration at the lowest treatment level in addition to issues associated with the accuracy of pore water measurements (discussed below). Overlying water mean measured concentration analysis is determined to be trivial as the testing apparatus ensures volume replacement three times weekly, and it is the sediment, not the overlying water, that is spiked with cyfluthrin.

Statistical differences in the analysis of this study were based on differences from the negative control, as per EFED guidance (Frankenberry *et al.*, 2008). In ascending order of the treatment levels (including the negative and solvent controls), the percent survival after 28 days was 79, 84, 86, 87, 84, 30, 0, and 0%. The three highest treatment levels (35, 60, and 290 ug a.i/kg sediment) showed statistically significant differences ($p<0.05$) from the negative control. The 28-day LC_{50} could not be determined as the probit method yielded results where the probability was less than 0.05 indicating poor goodness of fit. However, it was calculated using the moving average method and was determined to be 16.7 ug a.i/kg sediment (and 407 ug/kg TOC on an organic carbon-normalized basis). The 28-day NOAEC and LOAEC for survival and growth were determined to be 13 and 35 ug a.i/kg sediment, respectively (317 and 864 ug/kg TOC on an organic carbon-normalized basis). The EC_{50} for growth was determined to be >35 µg a.i/kg sediment based on less than a 50% reduction in growth at all treatment levels below this level

tested. Furthermore, the two highest treatment levels were excluded from the statistical analysis from the growth endpoint due to complete mortality in these treatment levels.

At test termination, statistical analysis showed no significant different between negative and solvent control growth. The study reviewer's statistical analysis based on effects on survival and growth of the negative control. Due to complete mortality at the two highest treatment levels (60 and 290 ug a.i/kg sediment), growth data was not available for these treatment levels.

This reviewer notes that HPLC analysis of cyfluthrin concentrations in porewater (conducted only at the highest test concentration) indicate that the parent material was only a small fraction of total radioactive residues measured over the course of this study (25 % to 0.73% for initial and terminal measurements, respectively). In contrast, the recovery of parent compound from bulk sediment was generally high (93% to 75% for initial and terminal measurements, respectively). Given that recovery of parent chemical was high based on QA/QC samples, the low concentrations of parent material in the porewater appear to reflect desorption of the degradation products from the sediment particles into the porewater phase. This presumption is consistent with the expected lower hydrophobicity of the degradation products compared to the parent compound. Given that the measured porewater concentrations of cyfluthrin do not accurately describe the exposure to parent compound, endpoints from this study will not be expressed in terms of measured porewater concentrations.

Instead, this reviewer has estimated freely dissolved porewater endpoints based on measured concentrations in bulk sediment, the fraction of total organic carbon in bulk sediment 4.1% and the mean Koc (124,000 mL/g-OC, MRID 00131495, 00137544, 45022103) for cyfluthrin. These estimated porewater endpoints, which are based on the freely dissolved test material (i.e., chemical that is not sorbed onto particulate organic carbon [POC] or dissolved organic carbon [DOC]), are consistent with the expression of aquatic estimated environmental concentrations (EECs) from PRZM/EXAMS. It is noted, however, that Koc values for cyfluthrin vary considerably (73,500 mL/g – 180,300 mL/g.-OC) which likely reflect differences in organic carbon composition and other soil properties used to determine Koc. Therefore, these estimated porewater endpoints are subject to the same uncertainty in determination and application of Koc for cyfluthrin.

This study was submitted to fulfill U.S. EPA data requirements for whole sediment chronic toxicity to estuarine/marine invertebrates based on "Methods for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-Associated Contaminants with the Amphipod *Leptocheirus plumulosus.*" Office of Research and Development, U.S. EPA. Washington, DC EPA/600/R-01/020 (2001). An LC_{50} for mortality and an EC_{50} for growth (dry weight) could not be determined by the reviewer as the data were unsuitable for statistical analysis using the Nuthatch or ICp programs. Even though the study follows test methods outlined by the document cited above, reproduction is a required endpoint, and was not assessed in this study. This study is

scientifically sound and still may be used in risk assessment for evaluation of effects of chronic exposure on growth and survival of *Leptocheirus.* It is classified as SUPPLEMENTAL.

Results Synopsis:

Based on mean-measured sediment concentrations (total radioactive residues):

Mortality:

LC_{50}: 16.7 µg a.i/kg sediment
NOAEC: 13 µg a.i/kg sediment
LOAEC: 35 ug a.i/kg sediment
95% C.I.: 14.5-19.4 µg a.i/kg sediment
Probit Slope: N/A

Growth (dry weight):

EC_{50} growth: >35 µg a.i/kg sediment
NOAEC: 13 µg a.i/kg sediment
LOAEC: 35 ug a.i/kg sediment
95% C.I.: N/A
Slope: N/A

Based on organic carbon-normalized mean-measured sediment concentrations (total radioactive residues):

Mortality:

LC_{50}: 407 µg a.i/kg TOC
NOAEC: 317 µg a.i/kg TOC
LOAEC: 854 ug a.i/kg TOC
95% C.I.: 354-473 µg a.i/kg TOC
Probit Slope: N/A

Growth (dry weight):

EC_{50}: >854 µg a.i/kg TOC
NOAEC : 317 µg a.i/kg TOC
LOAEC: 854 ug a.i/kg TOC
95% C.I.: N/A
Slope: N/A

Based on ESTIMATED[1] pore water concentrations (total radioactive residues):

Mortality:

LC_{50}: 0.0033
NOAEC : 0.0026 ug a.i/L
LOAEC: 0.0069 ug a.i/L
95% C.I.: 0.0029-0.0038 ug a.i/L
Probit Slope: N/A

Growth (dry weight):

EC_{50}: > 0.0069 ug a.i/L
NOAEC: 0.0026 ug a.i/L
LOAEC: 0.0069 ug a.i/L
95% C.I.: N/A
Slope: N/A

[1] Freely dissolved pore water endpoints (ug/L) estimated as:
Mean measured bulk sediment conc. (*ug/kg-dw*) / [Fraction TOC (*kg OC/kg-dw*) * K_{OC} (*L/kg-OC*)]

Endpoints affected: survival and growth
Most sensitive endpoint(s): Survival and growth (based on same NOAEC)

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: Rationale: Even though the study follows test methods outlined by the document cited above, reproduction is a required endpoint and was not assessed in this study.

C. Reparability: This study is not repairable as a new study will need to be conducted with reproduction as an endpoint.

9. MAJOR GUIDELINE DEVIATIONS: The following deviations from the above cited guidance methods were observed:

1. Neonate amphipods were acclimated and tested under differing temperatures. The acclimation temperature for 48 hours prior to test initiation was 17-19°C, and the testing temperature was 25 ± 1°C.

2. A physical description of the test substance was not provided. In addition, the aqueous solubility should have been reported.

3. In this study, negative control survival was 79%, slightly less than the minimum mean control survival of 80% recommended in Agency guidance. However, the difference is considered negligible, given the small magnitude between negative control survival and Agency guidance (1%). No single control replicate had less than 60% survival, another component of the Agency control performance criteria for chronic estuarine/marine benthic toxicity tests. In addition, the solvent control survival was 84%, higher than the Agency recommendation for control survival.

4. The study design did not include reproductive endpoints.

10. SUBMISSION PURPOSE: RED follow up

11. MATERIALS AND METHODS

Stability of Compound Under Test Conditions: [^{14}C]Residues were predominantly associated with the sediment, but declined 16-43% between test initiation and termination (reviewer-calculated). Mean percent recoveries of total radioactive residues (reviewer-calculated from LSC results) in bulk sediment were 51-90% of nominal concentrations on day 0, declining to 29-65%

of nominal on day 28. On days 0 and 28 at the 450 µg ai/kg level (the only level analyzed by HPLC/RAM), 93 and 75% of the recovered radioactivity from bulk sediment was parent material, respectively.

Less than an average of 7 µg/L was detected in the pore water during the study (based on LSC), and concentrations were generally consistent between 0 and 28 days. Mean recoveries increased from <0.039 µg/L (<LOQ) at the 1.9 µg/kg level to 6.9 µg/L at the 450 µg/kg level. Of the total radioactivity in porewater recovered from the 450 µg/kg level, 25% was identified as [^{14}C]cyfluthrin on day 0, and only 0.73% on day 28 (based on HPLC/RAM analysis).

Less than 2 µg/L was detected in the overlying water during the study (based on LSC), and samples were not further analyzed by HPLC/RAM.

Storage conditions of test chemical: At room temperature in the original container

Physicochemical properties of Cyfluthrin.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapour pressure	Not reported	
UV adsorption	Not reported	
pKa	Not reported	
Kow	Not reported	

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
Species	*Leptocheirus plumulosus*
Source	Laboratory cultures
Culture Conditions	Adult amphipods were maintained at 17-19°C in 11-L plastic bins containing a 2-cm layer of marine sediment and 7-8 L of 20‰ salinity seawater.
Age of Test Organisms	Neonates: size-selected (retained between 0.25 and 0.6-mm mesh screens)

Guideline Criteria	Reported Information
Food	During holding and acclimation, amphipods were fed daily a finely-ground suspension of Zeigler Prime flakes fish food (i.e., 100 mg/ml).
Health of parent culture stock	No mortality observed in the population 48 hours prior to test initiation.

B. Test System

Guideline Criteria	Reported Information
Type of Test System	Static-renewal
Test Water	Seawater was pumped from the Cape Cod Canal, Bourne, MA from about 4 m offshore at a depth of approx. 0.5 m. The seawater was filtered (not further specified) and adjusted to a salinity of 20-21‰ and a pH of 7.9 with laboratory well water.
Renewal of overlying water	3 times per week (Monday, Wednesday, and Friday), 400 ml of the overlying was siphoned off and replaced with fresh overlying water. Care was taken to not disturb the sediment layer.
Test Sediment	Marine sediment was collected from Little Harbor Beach, Wareham, MA. The sediment was wet pressed through a 0.25-mm sieve to remove large particles.
Sediment Characterization	Particle size: 68% sand, 19% silt, and 13% clay pH: 6.9 Ammonia (as N) in pore water: 6.0 mg/L TOC: 4.1% Percent water content (1/3 bar): 39% Grain size: 32% silt/clay

Guideline Criteria	Reported Information
Test Material	[^{14}C]Cyfluthrin Description: not reported Lot no.: 2003BRP-176-179 (reference no.) CAS No.: not reported Position of label: phenyl ring Radiochemical purity: 99% Specific activity: 121.9 mCi/mmole (623,113 dpm/µg) Storage: room temperature Aqueous solubility: Not reported. According to Laskowski (2002), the solubility is low, 2.3 ug/L or 2.3 ppb.
Solvents	Acetone, 9 ml per 0.6734 kg sediment (dw basis). The acetone was allowed to evaporate during the mixing procedure. Both solvent control and negative control groups were included in the study.
Sediment Spiking	A jar-rolling technique was used to apply the test substance to the sediment. An appropriate volume of each stock solution was applied to coarse silica sand and the solvent was allowed to evaporate off for 30 minutes. The sand was then added to 2.00 kg of wet sediment. Each jar was then rolled for 4 hours at room temperature at approx. 15 rpm. The jars were stored upright at 4°C overnight prior to conditioning.
Sediment Conditioning	The treated sediments were allowed to equilibrate for a 32-day period in the refrigerator. Once a week and prior to addition to the exposure vessels, the jars were mixed on the rolling mill for an additional 2 hours at room temperature to ensure the sediment was homogeneous.

Guideline Criteria	Reported Information
Sediment and Overlying Water Into Test Chambers	One day prior to the addition of amphipods (day -1), the test systems were established. Overlying water was gently added. 1 L glass vessels containing 175 ml (approx. 2.0 cm layer) of sediment (equivalent to 193 g wet weight or 60 g dry weight per vessel) and 725 ml of overlying water. The total overlying water plus sediment volume was maintained at approx. 900 ml. Test vessels were covered with a plastic plate. Nine replicates were prepared for each test concentration and control. Five replicates were used to evaluate the biological response and the remaining four were used for chemical analysis and water quality measurements.
Aeration	Test chambers were aerated with oil-free air (rate not reported). It was not reported if aeration was stopped during introduction of the test organisms.
Water Temperature	Overlying water: 24-27°C Pore water: not determined
pH	Overlying water: 7.2-8.1 Pore water: 6.7-7.3
Salinity	Overlying water: 20-21‰ Pore water: 20-21‰
Ammonia (as N)	Overlying water: 1.9-2.9 mg/L on day 0 and ≤0.35 mg/L on day 28 Pore water: 14.5-15.8 mg/L on day 0 and 3.5-5.9 mg/L on day 28
Dissolved Organic Carbon	Pore water: 14.1-49.5 mg/L
Dissolved Oxygen	5.4-6.9 mg/L (>60% saturation)

Guideline Criteria	Reported Information
Photoperiod	16 hours light, 8 hours dark (620-910 lux)
Food	Finely ground flaked fish food suspension (10 mg/ml). Amphipods were fed three times per week, following renewal of the overlying water. Days 0-13: 2 ml of suspension Days 14-27: 4 ml of suspension

C. Test Design

Guideline Criteria	Reported Information
Duration	28 days
Nominal Concentrations	Negative control, solvent control, 1.9, 5.6, 17, 50, 150, and 450 µg ai/kg dw sediment Selection of nominal treatment levels for the definitive study was based on results from preliminary testing.
Mean-Measured Concentrations	<0.15 (<LOQ; controls), 1.4, 3.8, 13, 35, 60, and 290 µg total [^{14}C]cyfluthrin residues/kg dw sediment (based on LSC analysis)
Number of Test Organisms	100 amphipods per level, divided into 5 replicates each containing 20 amphipods
Test organisms randomly or impartially assigned to test vessels?	Yes, organisms were impartially assigned to test containers.

Guideline Criteria	Reported Information
Overlying Water Parameter Measurements	Dissolved oxygen, salinity, temperature, and pH were measured daily in each control and treatment level; measurements were taken from all replicate chambers on days 0 and 28, and from alternating chambers on days 1-27. Temperature was also continuously monitored in one representative test vessel (solvent control, replicate I). Ammonia (as nitrogen) was measured on days 0 and 28 from a composite sample obtained for each control and treatment level.
Pore Water Parameter Measurements	Salinity, pH, ammonia, and dissolved organic carbon (DOC) were measured from a single replicate on days 0 and 28.
Chemical Analysis-Overlying Water	All control and treatment levels were analyzed on days 0 and 28 for total [^{14}C]residues using LSC.
Interstitial Water and Sediment Isolation Method	Centrifugation for 30 min at 10,000 *g*.
Chemical Analysis-Interstitial Water	All control and treatment levels were analyzed on days 0 and 28 for total [^{14}C]residues using LSC. In addition, samples from the 450 µg/kg level were analyzed for [^{14}C]cyfluthrin using HPLC/RAM.
Chemical Analysis-Bulk Sediment	All control and treatment levels were analyzed on days 0 and 28 for total [^{14}C]residues using LSC. In addition, samples from the 450 µg/kg level were analyzed for [^{14}C]cyfluthrin using HPLC/RAM.

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality	21% - negative control 16% - solvent control
Percent Recovery of Chemical:	Based on QC samples prepared and analyzed concurrently with sample analysis: LSC Sediment: 86.8-107% of nominal, with a single outlier of 139% Overlying water: 94.4-107% of nominal HPLC/RAM Sediment: 100% associated with parent Pore water: 99.1-99.6% associated with parent
Data Endpoints	- Survival - Dry weight
Observation Intervals	Daily for survival and abnormal behavior. Growth was determined from surviving organisms at day 28.
Raw data included?	Yes, mean replicate data provided

Effects Data (Reviewer-determined)

Toxicant Concentration [a]				Average Percent Survival, Day 28	Average Dry Weight/ Amphipod, mg
Nominal Sediment, µg/kg dw	Mean-measured Sediment, µg/kg dw	Mean-measured Pore Water, µg/L	Mean-measured, Overlying Water, µg/L		
Control	<0.15	<0.040	<0.016	79	1.71
Solvent Control	<0.15	<0.040	<0.016	84	1.58
1.9	1.4	0.019[d]	<0.016	86	1.84
5.6	3.8	0.093	<0.016	87	1.90
17	13	0.25	0.030 [b]	84	1.91
50	35	0.64	0.092	30*	0.93*
150	60	2.3	0.24	0*	--- [c]
450	290	6.9	0.83	0*	--- [c]

[a]) All mean-measured values were based on LSC results of total radioactive residues.
[b] Reviewer-calculated using ½ the LOQ for the day 0 result.
[c] Excluded from statistical analysis of growth due to complete mortality in these treatment levels
[d] Reviewer-calculated using ½ the LOQ for the days 0 and 28 results.
* Statistically different (≤0.05) compared to the negative control.

B. Statistical Results (From Study Report)

Endpoints analyzed were amphipod survival and growth (dry weight), both assessed on day 28 data. Analyses were performed with Toxstat Version 3.5 statistical software using the mean replicate organism response in each treatment group rather than individual response values. Survival data were arcsine square-root transformed prior to analysis.

For both endpoints, a t-Test was conducted to compare the performance of the negative and solvent control organisms. As no differences were observed, the data were pooled for subsequent comparisons. The data were then tested for normality using the Shapiro-Wilk's Test

and for homogeneity of variance using Levene's Test. Growth data were normally distributed and met the assumption for homogeneity, and were analyzed using Williams' Test to determine the NOAEC and LOAEC values. Survival data failed to meet the assumptions for normality and homogeneity, and were therefore analyzed using Wilcoxon's Rank Sum Test.

The Inhibition Concentration Method was used to calculate the 28-day LC/EC_{50} values with associated 95% confidence intervals.

Results were provided in terms of mean-measured sediment concentrations.

Study Author's Statistical Results

Endpoint	Methods	LC/EC_{50} (95% CI) (µg/kg)	NOAEC (µg/kg)	LOAEC (µg/kg)
Survival	ICp Wilcoxon's Rank Sum Test	35 (32-38)	13	35
Growth	ICp Williams' Test	36 (34-38)	13	35

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Data for survival and dry weight were tested using the Chi-square and Shapiro-Wilks tests for normality and the Hartley and Bartlett tests for homogeneity of variances. Data satisfied these assumptions, so the NOAEC and LOAEC were determined using this test, followed by William's multiple comparison test. For both endpoints, the solvent control data were compared to the negative control data using a Student's t-test. No significant differences were detected between the control groups for survival or dry weight; therefore, the negative control group was used for comparison to the treatment groups. These analyses were conducted using Toxstat statistical software. The 28-day LC_{50} was determined using the moving average method in the Toxanal (2009) software because of poor goodness of fit ($p<0.001$) using the probit method. Differences in the study author's and reviewer's LC_{50} values (35 and 16.7 µg a.i/kg sediment, respectively) likely reflect differences in the statistical method used (ICp vs. Moving Average). The EC_{50} for growth was determined to be >35 µg a.i/kg sediment based on less than a 50% reduction in growth at all treatment levels below this level tested. Furthermore, the two highest treatment levels were excluded from the statistical analysis from the growth endpoint due to complete mortality in these treatment levels. The reviewer expressed the NOAEC and LOAEC based on the mean measured sediment and pore water concentrations.

The above statistical analyses were performed in terms of the mean-measured sediment and estimated pore water treatment concentrations. Sediment endpoints are also reported on an OC-normalized basis, based on the following equation:

$$\text{mg/kg OC} = \frac{\text{mg/kg dry weight}}{\text{kg TOC/kg dry weight}}$$

This reviewer notes that HPLC analysis of cyfluthrin concentrations in porewater (conducted only at the highest test concentration) indicate that the parent material was only a small fraction of total radioactive residues measured over the course of this study (25 % to 0.73% for initial and terminal measurements, respectively). In contrast, the recovery of parent compound from bulk sediment was generally high (93% to 75% for initial and terminal measurements, respectively). Given that recovery of parent chemical was high based on QA/QC samples, the low concentrations of parent material in the porewater appear to reflect desorption of the degradation products from the sediment particles into the porewater phase. This presumption is consistent with the expected lower hydrophobicity of the degradation products compared to the parent compound. Given that the measured porewater concentrations of cyfluthrin do not accurately describe the exposure to parent compound, endpoints from this study will not be expressed in terms of measured porewater concentrations.

Instead, this reviewer has estimated freely dissolved porewater endpoints based on measured concentrations in bulk sediment, the fraction of total organic carbon in bulk sediment 4.1% and the mean Koc (124,000 mL/g-OC, MRID 00131495, 00137544, 45022103) for cyfluthrin. These estimated porewater endpoints, which are based on the freely dissolved test material (i.e., chemical that is not sorbed onto particulate organic carbon [POC] or dissolved organic carbon [DOC]), are consistent with the expression of aquatic estimated environmental concentrations (EECs) from PRZM/EXAMS. It is noted, however, that Koc values for cyfluthrin vary considerably (73,500 mL/g – 180,300 mL/g.-OC) which likely reflect differences in organic carbon composition and other soil properties used to determine Koc. Therefore, these estimated porewater endpoints are subject to the same uncertainty in determination and application of Koc for cyfluthrin.

Results Synopsis:

Based on mean-measured sediment concentrations (total radioactive residues):

Mortality:

LC_{50}: 16.7 µg a.i/kg sediment
95% C.I.: 14.5-19.4 µg a.i/kg sediment
NOAEC: 13 µg a.i/kg sediment
Probit Slope: N/A
LOAEC: 35 ug a.i/kg sediment

Growth (dry weight):

EC_{50} growth: >35 µg a.i/kg sediment 95% C.I.: N/A
NOAEC: 13 µg a.i/kg sediment Slope: N/A
LOAEC: 35 ug a.i/kg sediment

Based on organic carbon-normalized mean-measured sediment concentrations (total radioactive residues):

Mortality:
LC_{50}: 407 µg a.i/kg TOC 95% C.I.: 354-473 µg a.i/kg TOC
NOAEC: 317 µg a.i/kg TOC Probit Slope: N/A
LOAEC: 854 ug a.i/kg TOC

Growth (dry weight):
EC_{50}: >854 µg a.i/kg TOC 95% C.I.: N/A
NOAEC : 317 µg a.i/kg TOC Slope: N/A
LOAEC: 854 ug a.i/kg TOC

Based on ESTIMATED[1] pore water concentrations (total radioactive residues):

Mortality:
LC_{50}: 0.0033 95% C.I.: 0.0029-0.0038 ug a.i/L
NOAEC : 0.0026 ug a.i/L Probit Slope: N/A
LOAEC: 0.0069 ug a.i/L

Growth (dry weight):
EC_{50}: > 0.0069 ug a.i/L 95% C.I.: N/A
NOAEC: 0.0026 ug a.i/L Slope: N/A
LOAEC: 0.0069 ug a.i/L

[1] Freely dissolved pore water endpoints (ug/L) estimated as:
Mean measured bulk sediment conc. (*ug/kg-dw*) / [Fraction TOC (*kg OC/kg-dw*) * K_{OC} (*L/kg-OC*)]

Endpoints affected: survival and growth
Most sensitive endpoint(s): Survival and growth (based on same NOAEC)

14. REVIEWER'S COMMENTS:

The reviewer's conclusions for the NOAEC and LOAEC values were identical to those of the study author, but the reviewer was unable to determine a definitive EC_{50} value for growth. A 28-day EC_{50} for growth was determined to be >35 ug a.i/kg sediment based on less than a 50% reduction in growth at all treatment levels below this level tested. Furthermore, the two highest treatment levels were excluded from the statistical analysis for the growth endpoint due to complete mortality in these treatment levels. The NOAEC and LOAEC for this endpoint were also 13 and 35 µg a.i/kg dw sediment. Additionally, the study author estimated the LC_{50} using

the ICp method while the study reviewer used the Moving average method which likely accounts for the differences in values obtained from the two analyses.

In this 28-day sediment toxicity study, 400 uL of fresh dilution water (not spiked with test material) replaced 400 uL of previously added overlying water three times per week. Care was taken when siphoning the water off as to not disturb the sediment layer beneath the overlying water. Following replacement of the overlying water, the food ration for that day was added to each vessel. The Day 0 measured overlying water concentrations were <0.016 (<LOQ), <0.016, <0.016, <0.016, 0.043, 0.15, and 0.46 ug a.i/L while the Day 28 measured concentrations were <0.016, <0.016, <0.016, 0.052, 0.14, 0.32, and 1.2 ug a.i/L for the negative control and mean measured spiked sediment 2.2, 5.1, 13, 40, 125, and 383 ug a.i/kg dry sediment concentrations. The reviewer-determined mean measured overlying water concentrations were <0.016 (<LOQ), <0.016, <0.016, <0.034, 0.092, 0.12, and 0.83 ug a.i/L (average of the Day 0 and Day 28 measured concentrations). This particular type of test is designed to examine the effects of cyfluthrin to sediment dwelling organisms through pore water and sediment exposure, and the overlying water treatment concentrations are not the focus of this study.

For the definitive test (MRID 46591506), six individual dosing stock solutions were prepared in acetone for application to the test material to the sediment. These stock solutions were prepared using radiolabeled test material according to the following preparation scheme.

Conc. of Radiolabeled Stock Used (µg/mL)	Volume of Radiolabeled Stock Used (mL)	Diluted to Final Volume with Acetone (mL)	Dosing Stock Concentration (mg/mL)	Percent Radiolabeled (%)
102	8.22	25	33.7	100
33.7	3.33	10	11.2	100
33.7	1.11	10	3.74	100
33.7	0.378	10	1.27	100
33.7	0.125	10	0.421	100
33.7	0.042	10	0.142	100

All dosing stock solutions were clear and colorless with no visible undissolved test substance.

An appropriate amount (9 mL) of each individual dosing stock solution (above) was added to 0.0500 kg of course silica sand and placed in glass petri dishes. The solvent was allowed to evaporate for 30 minutes. The dry sand, containing the test material, was then added to the 2.0000 kg of wet sediment (0.6234 kg dry weight based on a percent of solids of 31.17%) in individual 1-gallon jars. The total mass of sediment spiked on a dry weight basis for each

treatment level and control was 0.6734 kg (0.0500 kg sand and 0.6234 kg dry weight sediment). The jars were sealed and rolled horizontally on a rolling mill for 4 hours at room temperature at approx. 15 rpm. Following the 4 hours of rolling, the jars were stored upright at 4°C overnight. The treated sediments were then allowed to equilibrate for 32 days in the refrigerator prior to allocation into the replicate test vessels. During the equilibration period, the treated sediments were rolled on the mill for an additional 2 hours once per week.

A 28-day preliminary test was conducted with non-radiolabeled cyfluthrin (purity of 93.3%) at nominal treatment levels of 0 (negative and solvent controls), 0.070, 0.70, 7.0, 70, and 700 µg ai/kg dw sediment. Three replicate vessels containing 20 amphipods each were exposed; otherwise, methods followed those described for the definitive study. After 28 days of exposure, 72, 87, 92, 63, and 0% survival was observed among amphipods exposed to the 0.070, 0.70, 7.0, 70, and 700 µg ai/kg treatment levels, respectively. In comparison, 87 and 75% survival was observed in the negative and solvent control groups, respectively. Dry weight among control amphipods averaged 1.20 and 1.09 mg for the negative and solvent control groups, respectively, compared to 1.23, 1.53, 1.01, and 0.70 mg for the 0.070, 0.70, 7.0, and 70 µg ai/kg treatment levels, respectively (100% mortality observed at the 700 µg ai/kg level).

This study was conducted in compliance with the U.S. EPA GLP regulations with the following exceptions: routine water, sediment and food contaminant screen analyses for pesticides, PCBs and toxic metals. Since the analyses were conducted following standard validated methods, these exceptions had no impact on the study results.

In-life dates were February 25 – March 25, 2005.

15. REFERENCES:

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

Survival:

Percent survival, ug/kg dw (sediment), Day 28
File: 1506ss Transform: NO TRANSFORMATION

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	79.0000	CALCULATED t VALUE =	-0.9623
GRP2 (BLANK CRTL) MEAN =	84.0000	DEGREES OF FREEDOM =	8
DIFFERENCE IN MEANS =	-5.0000		

TABLE t VALUE (0.05 (2), 8) = 2.306 **NO significant difference at alpha=0.05**
TABLE t VALUE (0.01 (2), 8) = 3.355 **NO significant difference at alpha=0.01**

Percent survival, ug/kg dw (sediment), Day 28
File: 1506ss Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	11854.000	2963.500	36.140
Within (Error)	20	1640.000	82.000	
Total	24	13494.000		

Critical F value = 2.87 (0.05,4,20)
Since F > Critical F **REJECT Ho:All groups equal**

Percent survival, ug/kg dw (sediment), Day 28
File: 1506ss Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	79.000	79.000		
2	1.4	86.000	86.000	-1.222	
3	3.8	87.000	87.000	-1.397	
4	13	84.000	84.000	-0.873	
5	35	30.000	30.000	8.556	*

Dunnett table value = 2.30 (1 Tailed Value, P=0.05, df=20,4)

Percent survival, ug/kg dw (sediment), Day 28
File: 1506ss Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL

1	neg control	5			
2	1.4	5	13.172	16.7	-7.000
3	3.8	5	13.172	16.7	-8.000
4	13	5	13.172	16.7	-5.000
5	35	5	13.172	16.7	49.000

Percent survival, ug/kg dw (sediment), Day 28
File: 1506ss Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	5	79.000	79.000	84.000
2	1.4	5	86.000	86.000	84.000
3	3.8	5	87.000	87.000	84.000
4	13	5	84.000	84.000	84.000
5	35	5	30.000	30.000	30.000

Percent survival, ug/kg dw (sediment), Day 28
File: 1506ss Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	84.000				
1.4	84.000	0.873		1.72	k= 1, v=20
3.8	84.000	0.873		1.81	k= 2, v=20
13	**84.000**	**0.873**		**1.83**	**k= 3, v=20**
35	30.000	8.556	*	1.85	k= 4, v=20

s = 9.055
Note: df used for table values are approximate when v > 20.

Dry Weight:
Mean dry weight (mg), ug/kg dw (sediment), Day 28
File: 1506sw Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	1.7160	CALCULATED t VALUE =	1.1260
GRP2 (BLANK CRTL) MEAN =	1.5840	DEGREES OF FREEDOM =	8
DIFFERENCE IN MEANS =	0.1320		

TABLE t VALUE (0.05 (2), 8) = 2.306 **NO significant difference at alpha=0.05**
TABLE t VALUE (0.01 (2), 8) = 3.355 **NO significant difference at alpha=0.01**

Mean dry weight (mg), ug/kg dw (sediment), Day 28
File: 1506sw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	3.426	0.857	42.850
Within (Error)	20	0.395	0.020	
Total	24	3.821		

Critical F value = 2.87 (0.05,4,20)
Since F > Critical F **REJECT Ho:All groups equal**

Mean dry weight (mg), ug/kg dw (sediment), Day 28
File: 1506sw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	1.716	1.716		
2	1.4	1.836	1.836	-1.342	
3	3.8	1.904	1.904	-2.102	
4	13	1.914	1.914	-2.214	
5	35	0.934	0.934	8.743	*

Dunnett table value = 2.30 (1 Tailed Value, P=0.05, df=20,4)

Mean dry weight (mg), ug/kg dw (sediment), Day 28
File: 1506sw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	5			
2	1.4	5	0.206	12.0	-0.120
3	3.8	5	0.206	12.0	-0.188
4	13	5	0.206	12.0	-0.198
5	35	5	0.206	12.0	0.782

Mean dry weight (mg), ug/kg dw (sediment), Day 28
File: 1506sw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	5	1.716	1.716	1.843

2	1.4	5	1.836	1.836	1.843
3	3.8	5	1.904	1.904	1.843
4	13	5	1.914	1.914	1.843
5	35	5	0.934	0.934	0.934

Mean dry weight (mg), ug/kg dw (sediment), Day 28
File: 1506sw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	1.843				
1.4	1.843	1.423		1.72	k= 1, v=20
3.8	1.843	1.423		1.81	k= 2, v=20
13	**1.843**	**1.423**		**1.83**	**k= 3, v=20**
35	0.934	8.796	*	1.85	k= 4, v=20

s = 0.141
Note: df used for table values are approximate when v > 20.

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

kgs cyfluthrin 28-d sediment tox

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
290	79	79	100	0
60	79	79	100	0
35	79	49	62.02531	0
13	100	16	16	0
3.8	100	13	13	0
1.4	100	14	14	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 27.45706

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	1.225188E-02	16.71358	14.49796	19.42748

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.7793937	19.23826	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.605652
95 PERCENT CONFIDENCE LIMITS = .1881298 AND 3.023175

INTERCEPT=-1.985864

LC50 = 17.25027
95 PERCENT CONFIDENCE LIMITS = 1.861686 AND 295.5347

LC25 = 6.557185
95 PERCENT CONFIDENCE LIMITS = 3.869113E-03 AND 22.10569

LC10 = 2.745463
95 PERCENT CONFIDENCE LIMITS = 3.309906E-06 AND 9.648454

LC05 = 1.630619
95 PERCENT CONFIDENCE LIMITS = 4.269105E-08 AND 6.647402
****

Program: Nuthatch Date: 1/31/11

Toxicity measurement for continuous endpoints, using weighted nonlinear regression, weighting proportional to predicted means.

Reference

R.D. Bruce and D.J. Versteeg. 1992. A statistical procedure for modeling continuous toxicity data. Env. Tox. and Chem. 11:1485-1494.

Input file: CYFLUGRW.TXT

Raw data:

Sediment toxicity - Cyfluthrin Dry Weight

```
In c:\nuthatch\CYFLUGRW.TXT : `Neg. control`
Interpreted as Dose = 0

CYFLUGRW.TXT : Sediment toxicity - Cyfluthrin Dry Weight
------------------------------------------------------------------------
Williams Test
------------------------------------------------------------------------
[One-Sided Test for Decrease, alpha =     0.050000 ]

Dose          Isotone      T-bar P-value  Significance
                Means
-----------------------------------------------------
0               1.84          .
1.4             1.84     -1.423    N.S.
3.8             1.84     -1.423    N.S.
13              1.84     -1.423    N.S.
35             0.934      8.796  <0.005       *

 "*"=Significant; "N.S."=Not Significant.

!!!Failure #3:  Data not suitable for probit model fit.

Criterion is 3 or more distinct isotone means.
```